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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Galloway, et al.	Examiner: Shahnan Shah, Khatol S.
Serial No.: 09/747,521	Group Art Unit: 1645
Filed: December 21, 2000	
For: **METHODS FOR PROTECTION AGAINST LETHAL INFECTION WITH *BACILLUS ANTHRACIS***	Attorney Docket No.: 22727/04079

Assistant Commissioner for Patents
Washington, D.C. 20231

Rule 1.132 Declaration of Dr. Darrell R. Galloway

Dear Sir:

I, Darrell Galloway, an inventor in the above-identified application, state as follows:

1. I received my Bachelor's Degree in Microbiology from California State University-Los Angeles in 1973. I received my Ph.D. in Biochemistry from the University of California at Riverside in 1978. Thereafter, I was a postdoctoral fellow in Immunology at Scripps Clinic and Research Foundation in La Jolla, California. In 1984, I became an Assistant Professor in the Department of Microbiology at the Ohio State University. Presently I am an Associate Professor in the Department of Microbiology at the Ohio State University.

2. Examples detailed in the above-described application show that compositions comprising polynucleotides that encode an immunogenic fragment of *B. anthracis* lethal factor (LF)(amino acid 9 through amino acid 252 of the mature, LF protein), alone or in combination with a polynucleotide that encodes a fragment of *B. anthracis* protective antigen (PA)(amino acid 175 through amino acid 735 of the mature PA protein) stimulate antibody production, protect against lethal anthrax toxin challenge, and protect against lethal anthrax spore challenge. These studies were performed in mice. The mouse model has been used by others to study induction of immunity against anthrax, and positive results in this animal model are believed to be a good first indicator of the test composition's ability to induce a protective response.

I and my co-inventor have now performed further studies in rabbits. Positive results in rabbits are also believed to be a good indicator of the ability of a test composition to induce a protective response against *B. anthracis* lethal toxin or *B. anthracis* spores.

In one of these studies, New Zealand White rabbits were injected intramuscularly (IM) with a eukaryotic expression plasmid comprising DNA encoding a fragment of the LF protein (i.e., pCLF4), or a eukaryotic expression plasmid comprising DNA encoding a fragment of the PA protein (i.e., pCPA) or both plasmids on days 0 and 28, then boosted with IM injections of full-length, mature, mutated LF protein, full-length, mature PA protein or a combination of LF + PA proteins on day 56. Sera from the rabbits was taken on day 84 and tested for antibodies specific for LF and PA by indirect ELISA. Figure 1, which is attached hereto, shows the results of this study. (See pre-challenge data for results. Post challenge results shows the levels of antibodies in these animals after they had been challenged with lethal doses of *B. anthracis* spores as described below) Panel A shows the titer of antibodies specific for PA. These data show that anti-PA antibodies were produced in the rabbits in response to injection with pCPA, but significantly more anti-PA antibodies were produced in response to injection with both pCPA + pCLF4. Panel B of Figure 1 shows the titer of antibodies specific for LF. These data show that anti-LF antibodies were produced in the rabbits in response to injection with pCLF4, but significantly more anti-LF antibodies were produced in response to injection with both pCLF4 and pCPA.

Approximately 1 year after the rabbits were given the final injection, they were challenged with lethal doses of *B. anthracis* spores (50 LD_{50} each). This was done by generating an aerosol of *B. anthracis* spores using a nebulizer designed to generate droplets of an approximate diameter of 1-2 μm. Such challenge closely mimics the conditions under which humans contract respiratory

anthrax. The rabbits were then observed for 28 days to determine survival. The results (Table 1, which is attached hereto) showed that rabbits that were administered eukaryotic expression plasmids encoding PA or PA and LF survived the full 28 days. Indeed, these animals are still alive. Rabbits administered the LF-encoding plasmid alone survived for 6 days after challenge. Control animals, given no plasmids, survived 2 days. These results confirm that compositions comprising a polynucleotide encoding LF, a polynucleotide encoding PA, and, particularly, a composition comprising a polynucleotide encoding LF and a polynucleotide encoding PA are useful for providing protection against challenge with *B. anthracis* LF toxin or *B. anthracis* spores. Subsequent studies with plasmids encoding full-length, mature, mutated LF protein or amino acid 9 through amino acid 252 of mature LF protein have shown that LF alone is protective in rabbits against challenge with *B. anthracis* spores and that protection is increased when the animals are also injected with plasmids encoding full-length, mature PA protein or amino acid 175 through amino acid 735 of mature PA protein.

3. The Examiner has stated that the nucleic acid compositions described in Leppla et al. (US Patent No. 5,591,631)(hereinafter "Leppla") anticipate the nucleic acid based immunogenic compositions recited in claims 23-24, 26-27, 31, 41, 42, and 44 of the above-identified application.

I have read Leppla. Leppla recites nucleic-acid based compositions that produce fusion proteins. Although the nucleic acid-based compositions of Leppla comprise a polynucleotide that encodes all or a portion of the LF protein and a promoter that is operably linked to such polynucleotide, the prokaryotic promoters that are used by Leppla are different functionally and in their sequence from the eukaryotic promoters recited in amended claims 23 and 24 of the above-described application. Similarly, the prokaryotic expression vectors that are used by Leppela to produce his fusion proteins are different from the mammalian (eukaryotic) expression vectors that are recited in the claims of the instant application. The nucleic acid compositions of Leppla are specifically designed to express the Leppla fusion proteins in prokaryotic hosts, while the nucleic acid compositions of the instant application are designed to express LF protein or fragments thereof and PA proteins or fragments thereof in the mammalian (eukaryotic) hosts that are to be immunized.

Leppla discloses prokaryotic transcriptional promoters such as the lactose, tryptophan, and beta-lactamase promoter systems as well as phage lambda promoters. (See

column 6 , lines 50-56 of Leppla) These are promoters that have been derived from prokaryotic bacteria and bacterial phages. Bacterial promoters do not initiate transcription of nucleic acids in mammalian (eukaryotic) cells. Bacterial (prokaryotic)promoters do not drive expression of the protein encoded by the polynucleotides to which such promoters are linked in mammalian (eukaryotic) cells.

In contrast, claims 23 and 24 of the instant application specifically recite eukaryotic promoters that drive expression of the LF protein (or an immunogenic fragment thereof) and the PA protein (or an immunogenic fragment thereof) in mammalian (eukaryotic) cells. Furthermore, the instant specification describes mammalian (eukaryotic) promoters such as the human cytomegalovirus (CMV) immediate-early enhancer promoter. (See page 9, lines 29-31 of the instant application.) Mammalian (eukaryotic) promoters, such as CMV, do not function in prokaryotic, i.e., bacterial systems.

Leppla also indicates that the hosts for his expression vectors are microbial hosts or procaryotic hosts, and that the prokaryotic expression vectors disclosed therein "will contain expression control sequences compatible to the host cell."(See column 6, lines 44-53 of Leppla.) In contrast, the eukaryotic control sequences disclosed in the plasmids of the present application contain viral and eukaryotic expression control systems that are compatible with eucaryotic or mammalian host cells. In addition to a viral promoter which is used to express recombinant protein in eucaryotic or mammalian cells, the plasmid shown in Figure 3 of the instant application comprises additional mammalian transcriptional regulatory sequences, namely the CMV intron A and SV40 late poly(A) sequences. These sequences facilitate mRNA processing and, thus, enhance gene expression. Such sequences play no role in prokaryotic gene expression and are not part of the nucleic acid compositions of Leppla. Therefore, in a variety of ways, the nucleic acid compositions of Leppla are different from the nucleic acid compositions described in the instant application.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

12/12/02
Date

Darrell R. Galloway
Darrell R. Galloway

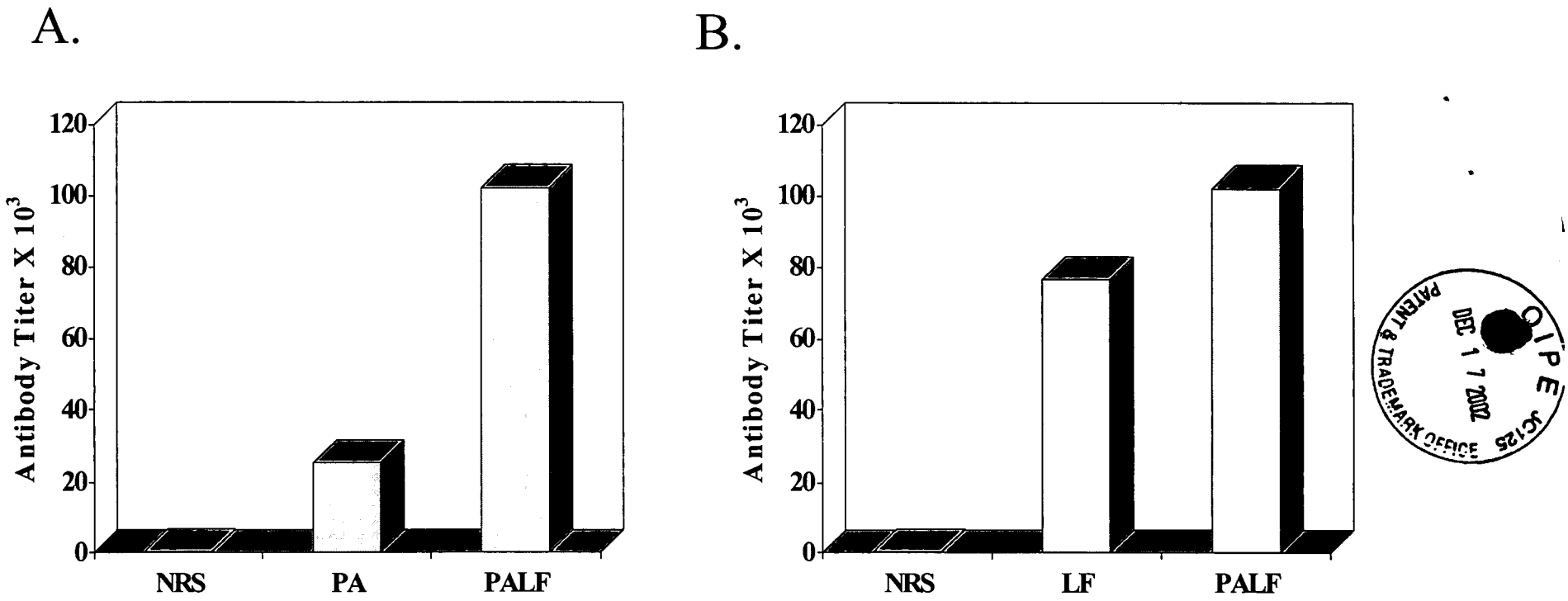

Figure 1. Serum antibody titers in New Zealand White rabbits following IM plasmid DNA immunization 2X via Biojector injection (Bioject, Inc.) with pCPA, pCLF4, pCPA + pCLF4, or NRS (negative control) followed by a single protein boost with PA_{83}, LF7, or PA_{83} + LF7, against purified protective antigen (A) or lethal factor (B).

Table 1.

Group	Anti-PA Concentration pre-challenge	post-challenge	Survival
2X DNA + 1X p.b.			
PALF4	9.8 ug/ml (1 yr)	192 ug/ml	Alive
PA	9.9 ug/ml (1 yr)	343 ug/ml	Alive
LF4	0 ug/ml	ND	6